Acetylcholinesterase inhibition, antioxidant and chemo-profile values of *Xylopia aethiopica* fruit and *Corchorus olitorius* leaf: preliminary neurological dysfunction protection evaluation

Ifeoma C. Orabueze[1,2], Gabriel O. Oludare[3], Esther. A. Adeyinfa[1], Taiwo M. Ghazali[4], Fortunia O. Orabueze[5], Gaber E. Batiha[6], George A. Asare[7]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria
[2]Department of African Center of Excellence for Drug Research Herbal Medicine Development and Regulatory Science (ACEDHARS), University of Lagos, Lagos, Nigeria
[3]Department of Physiology, Faculty of Basic Medical Sciences, University of Lagos, Nigeria
[4]Department of Pharmacognosy, Faculty of Pharmacy, University of Ilorin
[5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, PMB 12003, Surulere, Lagos, Nigeria.
[6]Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, AlBeheira, Egypt
[7]Department of Medical Laboratory Sciences, School of Biomedical and Allied Health Sciences, University of Ghana

Corresponding author: Ifeoma C. Orabueze
Email: iorabueze@unilag.edu.ng
Telephone: +234 808 133 2991; 234 803 343 5631

ABSTRACT

Background: *Xylopia aethiopica* fruit (XY) and *Corchorus olitorius* (COR) leaf are common food-herbs in Nigeria used in the management of convulsion and enhancement of learning.

Objective: The study aims to evaluate the cognitive dysfunction and neuro- protection profile of the 2 food-herbs and their solvent fractions.

Method: The phytoconstituent profiles of XY and COR were evaluated using Gas chromatography-Mass spectrometry (GC-MS). The antioxidant effects of the crude extracts were determined using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. An *in vitro* method, Ellman's colorimetric acetylcholinesterase inhibitory (AChE) assay was used to evaluate the cognitive enhancing/neuroprotective potentials of the plant crude extracts and fractions.

Results: The GC-MS study of *X. aethiopica* and *C. olitorius* extracts showed that some contained phytocompounds have been documented to possess anti-convulsive, sedative, anti-inflammation and hypnotic effects. The standard drug, eserine showed statistically higher antioxidant and acetylcholinesterase inhibitory effects compared to *C. olitorius* leaf and *X. aethiopica* fruit crude extracts. Only the AChE IC_{50}, obtained for *C. olitorius* ethyl acetate fraction (0.18 mg/mL) was lower than its crude extract value. While, the acetylcholinesterase inhibitory activities (IC_{50}), of all *X. aethiopica* fruit fractions were better than that of their crude extract.

Conclusion: The findings of this study suggest that the two nutritional food-herbs enhance cognitive ability and are possible agents in the management of degenerative impairments.

Keywords: Neuroprotection, *Xylopia aethiopica, Corchorus olitorius*, acetylcholinesterase inhibition cognitive impairment

Inhibition de l'acétylcholinestérase, activité antioxydante et profil chimique du fruit de *Xylopia aethiopica* et de la feuille de *Corchorus olitorius* : évaluation préliminaire de la protection contre les dysfonctionnements neurologiques

Ifeoma C. Orabueze[1,2], Gabriel O. Oludare[3], Esther. A. Adeyinfa[1], Taiwo M. Ghazali[4], Fortunia O. Orabueze[5], Gaber E. Batiha[6], George A. Asare[7]

[1]Département de pharmacognosie, Faculté de pharmacie, Université de Lagos, Lagos, Nigéria
[2]Département du Centre d'excellence africain pour la recherche sur les médicaments, le développement de la médecine traditionnelle et les sciences réglementaires (ACEDHARS), Université de Lagos, Lagos, Nigéria
[3]Département de physiologie, Faculté des sciences médicales fondamentales, Université de Lagos, Nigéria
[4]Département de pharmacognosie, Faculté de pharmacie, Université d'Ilorin
[5]Département de chimie pharmaceutique, Faculté de pharmacie, Université de Lagos, PMB 12003, Surulere, Lagos, Nigéria.
[6]Département de pharmacologie et de thérapeutique, Faculté de médecine vétérinaire, Université de Damanhour, Damanhour 22511, Al Beheira , Égypte
[7]Département des sciences de laboratoire médical, École des sciences biomédicales et paramédicales, Université du Ghana

Auteur correspondant: Ifeoma C. Orabueze
Courriel: iorabueze@unilag.edu.ng
Télephone: +2348081332991 ; +2348033435631

RÉSUMÉ

Contexte: Le fruit de *Xylopia aethiopica* (XY) et la feuille de *Corchorus olitorius* (COR) sont des herbes alimentaires courantes au Nigeria utilisées pour la prise en charge des convulsions et l'amélioration des capacités d'apprentissage.

Objectif: Cette étude vise à évaluer les troubles cognitifs ainsi que le profil neuroprotecteur de ces deux plantes alimentaires et de leurs fractions obtenues par solvants.

Méthode: Les profils phytochimiques de XY et COR ont été analysés par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS). Les effets antioxydants des extraits bruts ont été déterminés par le test de piégeage du radical 1,1-diphényl-2-picrylhydrazyle (DPPH). L'inhibition de l'acétylcholinestérase (AChE) a été évaluée in vitro par le test colorimétrique d'Ellman afin de déterminer les potentiels d'amélioration cognitive et de neuroprotection des extraits bruts et des fractions de la plante.

Résultats: L'analyse GC-MS des extraits de *X. aethiopica* et *C. olitorius* a révélé la présence de certains composés phytochimiques déjà documentés pour leurs propriétés anticonvulsivantes, sédatives, anti-inflammatoires et hypnotiques. Le médicament de référence, l'ésérine, a présenté des effets antioxydants et inhibiteurs de l'acétylcholinestérase statistiquement supérieurs à ceux des extraits bruts de feuilles de *Cyprinus olitorius* et de fruits de *Xanthomonas aethiopica*. Seule la valeur CI_{50} de l'AChE, obtenue pour la fraction d'acétate d'éthyle de *Cyprinus olitorius* (0,18 mg/mL), était inférieure à celle de l'extrait brut. En revanche, toutes les fractions de fruits de Xanthomonas aethiopica présentaient une meilleurs activité inhibitrice de l'acétylcholinestérase (IC_{50}) par rapport à leur extrait brut.

Conclusion: Les résultats suggèrent que ces deux plantes alimentaires possèdent des propriétés d'amélioration cognitive et pourraient constituer des agents potentiels dans la prise en charge des troubles neurodégénératifs.

Mots clés: Neuroprotection, *Xylopia aethiopica, Corchorus olitorius*, inhibition de l'acétylcholinestérase, troubles cognitifs

INTRODUCTION

Neurodegenerative diseases (ND) include convulsion, epilepsy, memory loss/cognitive dysfunction, psychosis, Alzheimer diseases (AD), parkinson disease, psychological disorders etc. They all have a common presentation of a progressive destruction of the nervous system that gradually affects the structural and functions of the brain. Neuronal degeneration, memory and cognitive loss in ND are irreversible, resulting in social stigmatization, social exclusion, and dependency.[1,2] Many factors such as, oxidative stress, brain injury, mitochondria dysfunction, neuroinflammation, abnormal protein aggregation in the neuronal, tissues and cholinergic dysfunction have been cited as major leading causes of neuronal damage.[3]

Memory and cognition dysfunction are symbolized by the decline in ability to remember or engage in thinking process with little or no difficulties. They are progressive neurodegenerative disorders associated with aging, specific genes and some disease states. Cognitive impairment and convulsion are hidden burden of malaria infection. The Plasmodium parasite load is detected and treated while the neurological effects are unrecognized and thus, left untreated. Report has shown that 5 and 26% of children exposed to severe symptomatic and asymptomatic falciparum malaria infection are challenged with cognitive impairment.[4] This affects their language, school performance, memory, behavioural character, causes neurologic abnormalities (febrile and non-afebrile convulsions, seizures, altered mental status, drowsiness, coma in very severe cases) and a challenged adult life.[5]

Medicinal plants are known to contain bioactive secondary metabolites that exhibit multi-target curative and prophylactics management of various human diseases. Plants have history of long use as healing agents and food supply. The chemical constituents of these plants have played vital roles in the discovery of drugs and drug leads. These medicinal plants are prepared in as simple ways as cooking, maceration (soaking the plant materials), burning, taking raw, brewing in forms of teas etc. These are done with the intention to release the bioactive constituents needed for the pharmacological activities. Medicinal plants are the bedrock of many clinical active drugs.

Nigeria has a good diversity of flora which can serve as source of therapeutic agents, food and wellness. The average Nigerian family feeds on meals that contain spices and vegetable sourced from various plants. These food aids do not only add flavour, taste and fillings to the table but could have a supportive therapeutic effect. When taken in the right quantity according to the daily needs of the body, they can provide phytoconstituents that could be protective and curative. Neuroprotective activities of many Nigerian plants have been reported and documented.[6,7] These plants could be sources for search of bioactive compounds with cholinesterase inhibitory potentials.

The detection of acetylcholine dysfunction signals indicates neuronal damage, which, if not managed appropriately, may lead to neurodegeneration and related diseases. Acetylcholinesterase (AChE) is an enzyme that breaks down acetylcholine (ACh) into choline and acetic acid and thus facilitates the termination of nerve impulse transmission at cholinergic synapses.[8,9] Physostigmine (eserine), is an example of plant sourced compound from Calabar bean seeds (Physostigma venenosa Balf.). It has been used clinically and experimentally to probe the alleviation of symptoms of Alzheimer diseases (AD) by improving cholinergic activity in the brain.[8,10] It exhibits AChE inhibitory effect but has limited use due to short half-life and several adverse effects. Rivastigmine is a derivative of natural sourced physostigmine and also clinically active. Donepezil, galantamine, tacrine and rivastigmine are acetylcholinesterase (AChE) inhibitor drugs approved by Food and Drug Administration (FDA) for use in the mitigation and management of AD and other memory affecting neurological diseases.[11-12] Thus, the search for more effective AChEi lead compounds from plants known to be used in traditional medicine for memory enhancing and anticonvulsive effect continues.

Xylopia aethiopica (Dunal) A Rich (Anonaceae) fruit is a common spice in Nigeria kitchens and traditional medicine due to its many ethnomedicinal, flavouring and food preservative effects.[13-15] It is a common plant in various regions of West Africa and identified in commercial places as African pepper, Guinea pepper, spice tree, West African pepper, Ethiopian pepper and Senegal pepper.[13-15] Across the Nigeria nation, it is used in preparation of age longed traditional nutritious concoctions for postpartum care for nursing mothers.[16] The spicy meals prepared from the plant fruits are well used during recovery from illness.[16] It is said in the Igbo tribe of Nigeria that it "breaks" fever and quickens recovery. Other ethnomedicinal uses of the seed/fruit include cough recipe, carminative, taste masking in herbal product preparation, treatment of diarrhoea,

dysentery stomach disorder, menstrual disorder, arthritis, rheumatism.[17]

Tossa jute sprout (*Corchorus olitorius*, Malvaceae) is an edible leafy vegetable used in making soup and other local dishes in Nigeria. In Nigeria, it is called ewedu and arira by the Yoruba and Igbo tribes respectively. And in the commercial international market, it is commonly known as bush okra, wild okra, Jew's mallow, tossa jute, long fruited jute, Meloukia, molokhia, Moroheia, Moroheiya, Mulukhiyah and Tasso.[18-20] The folkloric use of the leaves include treatment for fever, dysentery, enteritis, aches and pains, pectoral pains, diuretic, lactagogue, purgative and tonic properties. piles, pains, tumors, fever, gonorrhea dysuria.[18] The cold infusion of the leaves that is taken as cold tea has been reported to revive strength and appetite.[21] The leaf decoction is used as an emergency treatment for iron and folic acid deficiency and infantile malnutrition.[19,22] *Corchorus olitorius* has been scientifically shown to possess pharmacological diverse plant antioxidant, anti-inflammatory, hepatoprotective, antihyperlipidemic, immunostimulant, antitumor, antimicrobial, antidiabetic, analgesic, wound-healing properties and cardioprotective activities.[22,23]

Xylopia aethiopica fruit (XY) and *C. olitorius* (COR) are cultural and constant food items on Nigerian tables on daily basis, affordable, accepted and available. They are also believed to be food of wellness and used in feeding the sick and the recovering individuals. The study aimed to evaluate the AChE inhibitory potentials of XY and COR towards evaluating their local use as memory and cognitive enhancer. The antioxidant and phytoconstituent profiles of these plants were studied too. Both their crude extracts and solvent fractions were evaluated for these activities. Ellman's colorimetric method is a very rapid assay for the detection and quantification of AChE inhibitory ability in samples.[24,25]

MATERIALS AND METHODS

Chemicals/Materials

Acetylthiocholine iodide (ATChI), electric eel acetylcholinesterase (EC 3.1.1.7, type-VI-S) and 5,5?-dithio-bis-nitro- benzoic acid (DTNB) were bought from the Sigma. Buffers and other chemicals were of extra pure analytical grade. Physostigmine (eserine) was used as the standard drug.

Sample collection

The fruits of *X. aethiopica* were purchased from main market, Ile-epo market, Iyana ipaja Lagos State, Nigeria while the leaves of *C. olitorius* were collected from a farmland in Igando-Egan, Lagos state. The plants were identified and authenticated by a taxonomist, Mr. Felix Nwafor, of international center for ethnomedicine and drug development (InterCEDD), Nsukka, Enugu state. The herbarium specimen was prepared and deposited with voucher number.

Table 1: Biodata of the plants

Botanical name	Voucher Number
Xylopia aethiopica (Dunal) A. Rich.	UNN/11777
Corchorus olitorius L.	UNN/11778

Sample preparation

The samples were air dried, reduced into coarse powder (1 kg) using a grinding mill (Hamburg 76 West Germany) and extracted with 80 % ethanol by cold maceration (2000 mL) for 72 h. This was filtered on day 3 and re-soaked for another 2 days and filtered again. The filtrates were pooled together and concentrated under vacuum using a rotary evaporator (Buchi Vacuum module V-801 Easy Vac) at 45 rpm and 40 ºC to obtain the semi-solid crude extract. The drying was completed in a water bath at the temperature of 40 ºC. The dried extracts of *X. aethiopica* yielded (160.5 g (crude extract) and *C. olitorius* yielded (140.21 g (crude extract) and were stored in a refrigerator at 4 ºC in airtight plastic containers until used.

Fractionation

The crude extract of XY (130 g) was suspended in 400 mL of distilled water: methanol (2:1 ratio) and extracted with n-hexane (3 × 400 mL); dichloromethane (3 × 400 mL) and ethyl acetate (3 × 400 mL). The fractions were collected separately, dried by rotary evaporator at 40 ºC and stored at 4 ºC throughout experiments. The dried weights were:

n-hexane (XY-Hex, 19.25 g), dichloromethane (DCM-XY, 17.50 g), ethyl acetate (EA-XY, 15.35 g) and the aqueous (AQ-XY, 13.34 g).

The same was repeated for *C. olitorius* crude extract. The collected fractions were dried and weighed. The obtained dried weights were n-hexane (COR-Hex, 15.38 g), dichloromethane (DCM-COR, 14.66 g), ethyl acetate (EA-COR, 12.20 g) and the aqueous (AQ-COR, 10.60 g).

Phytochemical analysis

The phytochemical analysis of the constituents of the 2 extracts and their fractions were done using the standard procedures.[26]

Gas chromatography-mass spectrometry (GC-MS) profile crude extract

The crude extracts of *X. aethiopica* and *C. olitorius* (XY-CE and COR-CE respectively) were subjected to qualitative and quantitative analysis of their constituents using GC-MS technology. The evaluation was carried out with Agilent Technologies 7890 USA, equipped with MS detector 5975 Agilent Technologies and HP5 MS capillary column (30.00 m × 0.320 mm inner diameter × 0.25 µm film thickness). The start oven temperature was set at 80 ºC and increased to 240 ºC. The initial temperature was held for 2 minutes and gradually moved higher at the rate of 10 ºC/min. At the 240 ºC, the temperature was held for 6 minutes. The constant flow speed of the carrier gas helium, was 2 mL/min. The injected volume of test sample was 1 µL with a split-less mode. The GC-MS detection of the volatile phytoconstituents of the test samples were identified based on the comparison of peaks and retention time, literature reports and computer evaluation of mass spectra compared with standard spectra, National Institute Standard and Technique (NIST 2014 version 2.1.0).

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging assay

The DPPH scavenging effects of the *X. aethiopica* and *C. olitorius* (XY and COR) hydro-ethanol crude extracts were assayed using Mensor *et al.*[27] method but with slight modifications. A serial diluted solutions (10, 25, 50, 100, 250 µg mL^{-1}) of the crude extracts of XY and COR were prepared in methanol. To 1 mL of each of the prepared serial solutions, 3 mL of methanol and 1 mL, 1 mM DPPH solution were added to make up to a final volume of 5 mL. The resulting solutions were thoroughly mixed and kept in a dark room for 30 minutes to incubate. Vitamin C (ascorbic acid) was used as the standard positive control drug. The negative control was a blank solution of methanol (no extract added). Absorbance of the resulting serial solutions were recorded at wavelength of 517 nm. Measurement of values and adsorptions were made in triplicates.

Anticholinesterase assays

The anti-cholinesterase, acetylcholinesterase (AChE) inhibiting activities of the hydro-ethanol crude extracts and their solvent fractions were determined by using a modified method of Ellman *et al.*[24] as described by Jung *et al.*[28.] The extracts and the fractions were prepared in a stock solution of 5% Tween 20 in buffer and were used for the cholinesterase inhibition assay. Serial dilutions of the test samples (crude extracts and the fractions) were made to obtain varying concentrations of the test samples (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/mL). Eserine (Physostigmine hemisulfate salt), used as the standard drug here was prepared in buffer into varying concentrations of 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/mL.

Procedures

In a 96-well plate were added 140 µL of Phosphate buffer (100 mM, pH 8.0,), 20 µL of varying concentrations of the test samples (1, 0.5, 0.25, 0.125 0.0625 and 0.03125 mg/ml), and 20 µL of the enzyme (AChE, 0.28 µg/mL). The reaction mixtures were incubated for 15 minutes t 37 ºC, after which 10 µL of 10 mM DTNB was added. The reaction was initiated by the addition of 10 µL of 25 mM ATChI. The rate of hydrolysis of ATChI was then determined spectrophotometrically by measuring the change in the absorbance per minute (ΔA/min) due to the formation of the yellow 5-thio-2-nitrobenzoate anion at 412 nm over a period of 15 minutes at 1 minute interval. A solution of buffer was used as negative control in place of sample. All assays were carried out in triplicates.

Eserine (-) physostigmine) was used as positive control.

The percentage inhibition (%I) of the test sample was obtained using the formula:

$$I\ (\%) = [(Vo - Vi) / Vo] * 100 \quad \ldots\ldots 1$$

Where: I (%) = Percentage inhibition
Vi = enzyme activity in the presence of the test sample
Vo = enzyme activity in the absence of the test sample.

The enzyme activity was calculated as follows:

$$\text{Activity (µ moles/ min/ ml)} = \frac{\Delta A}{\text{min}} X \frac{v}{(\varepsilon \times v \times d)} \quad \ldots\ldots 2$$

Where: ΔA/ min = change in absorbance/minute
V = total volume of reaction mixture
v = volume of test sample in reaction mixture
ε = extinction coefficient of DTNB = 1.36 x 10 4
d = light path length (1 cm).

Half-maximal inhibitory concentration) (IC_{50}) values

The IC_{50} values, (concentrations that inhibits the activities of the crude extracts and the fractions by 50 %) were calculated using the Dose-response equation gotten from the dose-effect curves.

Data analysis

Data are given as the mean ± SEM. Experiments results were evaluated using analysis of variance (ANOVA). Dunnett's (post-hoc test) was used to determine statistical significance of means and $p < 0.05$ was regarded as statistically significant. Dose response equation was generated using the percentage inhibition and concentration of each extract. The IC_{50} values were calculated via linear regression analysis from dose-effect curves.

RESULTS

Phytochemical profile

The phytochemical constituents of *X. aethiopica* and *C. olitorius* hydro-ethanol crude extracts and their fractions are as shown in Tables and 3 respectively. The phytochemical tests of the 2 plants revealed the presence of triterpenes/sterols, alkaloids, flavonoids, and saponins in the various fractions.

Table 2: The Phytochemical constituents of the crude extract and solvent fractions of *X. aethiopica*

Phytochemicals	Crude extract	Hex	DCM	EA
Alkaloids	+	+	+	+
Terpenoids	+	-	+	+
Phenols and tannins	-	-	+	+
Cardiac glycosides	+	+	-	-
Saponins	+	+	+	+
Flavonoids	+	-	+	+
Anthraquinones	+	+	+	-

Key: - Absence + Presence Hex: Hexane fraction EA: Ethyl acetate fraction DCM: Dichloromethane fraction

Table 3: The Phytochemical constituents of the solvent fractions of *C. olitorius* hydro-ethanol crude extract

Phytochemicals	Crude extract	Hex	DCM	EA
Alkaloids	++	+	+	+
Terpenoids	+	+	-	+
Phenols and tannins	+	_-	+	+
Cardiac glycoside	+	+	-	-
Saponins	+	-	+	-
Flavonoids	+	-	+	+
Anthraquinones	-	-	-	+

Key: - Absence + PresenceHex: Hexane fraction DCM: Dichloromethane fractionEA: Ethyl acetate fraction *C. olitorius* hydro-ethanol crude extract showed a high-level content of alkaloid

Antioxidant result

Figure 1 shows the percent inhibition of DPPH radicals (scavenging effect) of *X. aethiopica* and *C. olitorius* hydro-ethanol crude extracts and the reference antioxidant compound (ascorbic acid) at the different serial diluted concentrations. The two hydro-ethanol extracts showed poor antioxidant values compared to the references drug, ascorbic acid. The DPPH scavenging ability of ascorbic acid is significantly ($p < 0.05$) stronger than the extracts. The free radical scavenging DPPH showed a concentration-dependent pattern for both extracts and the standard (ascorbic acid). The IC_{50} values of hydro-ethanol XY-CE and COR-CE and ascorbic acid were calculated to be 812.5, 426.8 and 0.001938 µg mL^{-1}, respectively. The DPPH scavenging strength of both extracts increased with concentration in a strongly linear manner (R^2 = 0.9624 and 0.9934 for XY-CE and COR-CE respectively). The DPPH free radical scavenging power of the ascorbic acid was R^2 =0.9472.

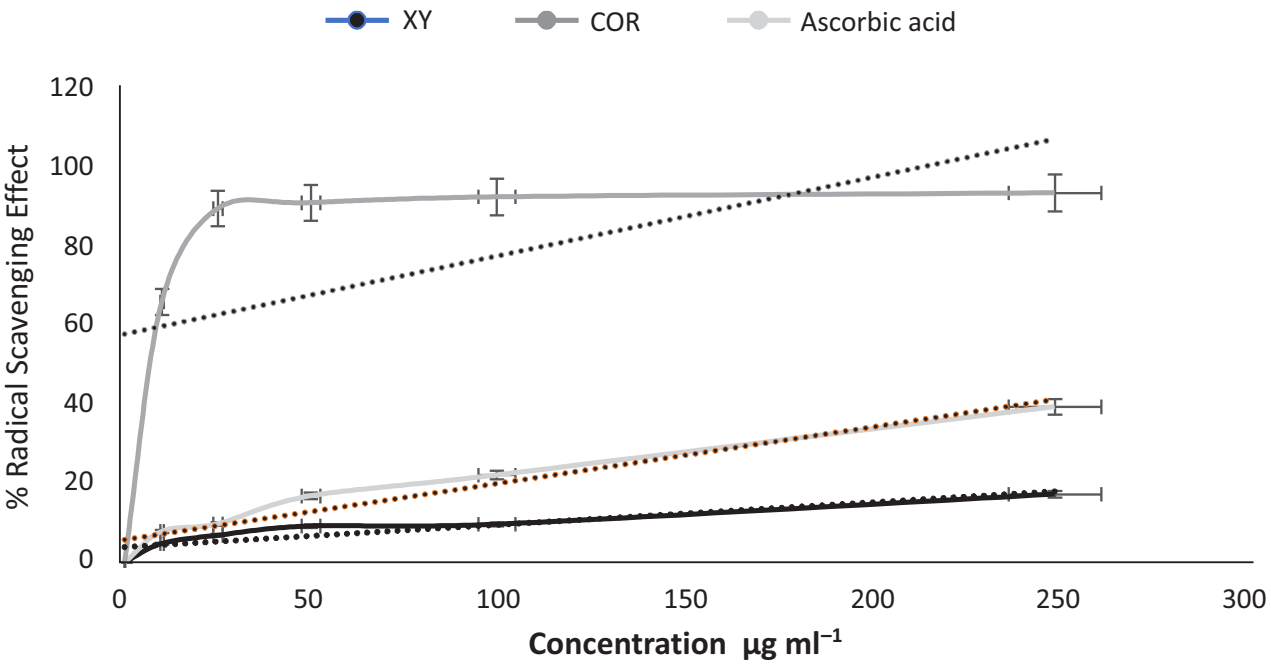

Fig. 1: DPPH radical scavenging ability of *C. olitorius* leaf extract, *X. aethiopica* fruit extract and Vit. C/Ascorbic acid (standard) Data are means ± SEM of triplicate experiments

GC-MS analysis results

The GC-MS analysis results of extracts of XY-CE and COR-CE are presented in Tables 4 and 5 respectively. The GC-MS spectra of XY-CE and COR-CE showed 53 and 48 peaks respectively indicating different phytochemical compounds. For XY-CE, only the phytoconstituents up to 1 % of the total and above are presented as shown in Table 4. While the phytochemical constituents of the COR-CE presented here are only those compounds up to 0.7 % of total and above, as shown in Table 5.

The 5 constituents identified from the XY-CE with the highest percentage peak areas include Nordextromethorphan(1R,4aR,4bS,7R,10aR)-1,4a,7-Trime (4.99 %); 1-Hydroxy-3-(octanoyloxy)propan-2-yl decanoate (5.96 %); 1-[P-Bromophenyl]-4-nitro-1,3-butadiene (7.46 %); 3, alpha., 17. beta-dihydroxyestr-4-ene (8 %) and 1,1,4,7-Tetramethyldecahydro-1H-CY clopropa[e]azulene-4,7-diol (17.64 %). The 5 compounds with the highest contents for COR include, 9,12,15-Octadecatrienoic acid, met hyl ester, (Z,Z,Z)- (4.5 %), n-Hexadecanoic acid (4.65 %), Propanoic acid, 2-methyl-, octyl ester (5.34%) and 3-Dibenzofuranamine (20.32%)

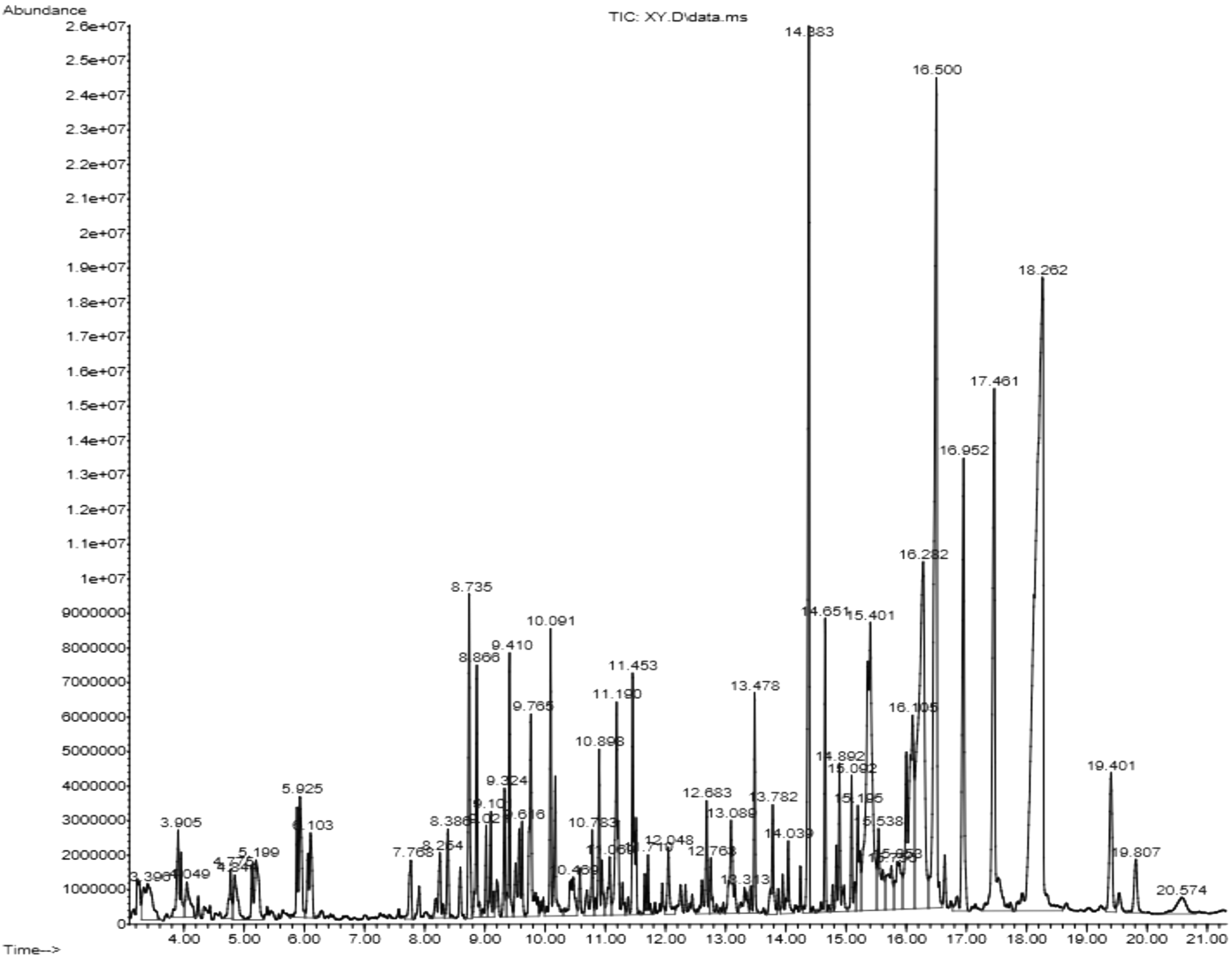

Fig. 2: GC-MS chromatogram of hydroethanol extract of *Xylopia aethiopica* fruit showing the retention time (min) of the compounds in X axis and percentage (%) of peak area in the Y axis

Table 4: GC-MS chemical profile of various fractions of *Xylopia aethiopica* fruit extract showing compound up to 1 % of total and above only

Peak Number	Compound	Molecular Formular	Mol. weight (g/mol)	Retention. Time (min)	% of Total
1	Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	$C_{10}H_{16}$	136.0	3.396	1.12
2	(1R) -2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	$C_{10}H_{16}$	136.0	3.905	1.04
3	2,4,6-Octatriene, 2,6-dimethyl-,E,Z)-	$C_{10}H_{16}$	136.0	5.199	1.16
4	Terpinen-4-ol	$C_{10}H_{18}O$	154.0	5.925	1.61
5	alpha.-Terpineol	$C_{10}H_{18}O$	154.0	6.103	1.03
6	Alfa.-Copaene	$C_{15}H_{24}$	204.0	8.254	0.65
7	beta.-ylangene	$C_{15}H_{24}$	204.0	8.735	1.80
8	(1R,2S,6S,7S,8S)-8-Isopropyl-1-met	$C_{15}H_{24}O$	220.0	8.866	1.37
9	(1R,2S,6S,7S,8S)-8-Isopropyl-1-met	$C_{15}H_{24}O$	220.0	9.410	1.26
10	1H-Cyclopenta [1,3] cyclopropa[1,2] b	$C_{15}H_{24}$	204.0	9.616	1.24
11	1-Isopropyl-4,7-dimethyl-1,2,3,5,6 ,8a-hexahydronaphthalene	$C_{15}H_{24}$	204.0	9.765	1.79
12	Cyclohexanemethanol, 4-ethenyl-.al	$C_{15}H_{26}O$	222.0	10.091	2.19
13	1,7,7-Trimethyl-2-vinylbicyclo [2.2 .1]hept-2-ene	$C_{12}H_{18}$	162.0	10.898	1.20
14	2-Naphthalenemethanol, 1,2,3,4,4a,	$C_{15}H_{26}O$	222.0	11.190	1.94
15	1,3,6-Heptatriene, 2,5,5-trimethyl	$C_{10}H_{16}$	136.0	11.453	1.85
16	9-Isopropyl-1-methyl-2-methylene-5 -oxatricyclo[5.4.0.0(3,8)]undecane	$C_{15}H_{24}O$	220.0	13.089	1.17
17	Pentadecanoic acid, 14-methyl-, ester	$C_{16}H_{32}O_2$	256.0	13.478	1.07
18	1H-Naptho [2,1-b] pyran 3-ethenyld odecahydro-3,4a,7,7,10a-pentamethyl-,	$C_{20}H_{34}O$	290.0	14.383	4.66
19	Kaur-16-ene	$C_{20}H_{32}$	272.0	14.651	1.34
20	7- Octadecenoic acid, methyl ester	$C_{19}H_{36}O$	296.0	14.892	1.32
22	1-Hydroxy-3-(octanoyloxy) propan-2-yl decanoate	$C_{12}H_{40}O_5$	372.0	15.401	5.96
23	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl-	$C_{15}H_{26}O$	222.0	15.538	1.08
24	3.5-Octanedione, 2,2,7-trimethyl	$C_{11}H_{20}O_2$	184.0	15.853	1.00
25	Octanoyl chloride	$C_8H_{15}C_{10}$	162.0	16.105	4.11
26	1-[P-Bromophenyl]-4-nitro-1,3-butadiene	$C_{10}H_9NO_2$	175.0	16.282	7.46
27	3, alpha., 17. beta-dihydroxyestr-4-ene	$C_{19}H_{30}O_2$	290.0	16.500	8.00
28	3-Hexadecyne	$C_{16}H_{30}$	222.0	16.952	3.55
29	Nordextromethorphan(1R,4aR,4bS,7R,10aR) -1,4a,7-Trime	$C_{20}H_{30}O$	286.0	17.61	4.99
30	1,1,4,7-Tetramethyldecahydro-1H-CY clopropa[e]azulene-4,7-diol	$C_{12}H_{26}O$	222.0	18.262	17.64
31	Pyridine, 2,4,6-trimethyl-	$C_8H_{11}N$	121.0	19.401	1.38

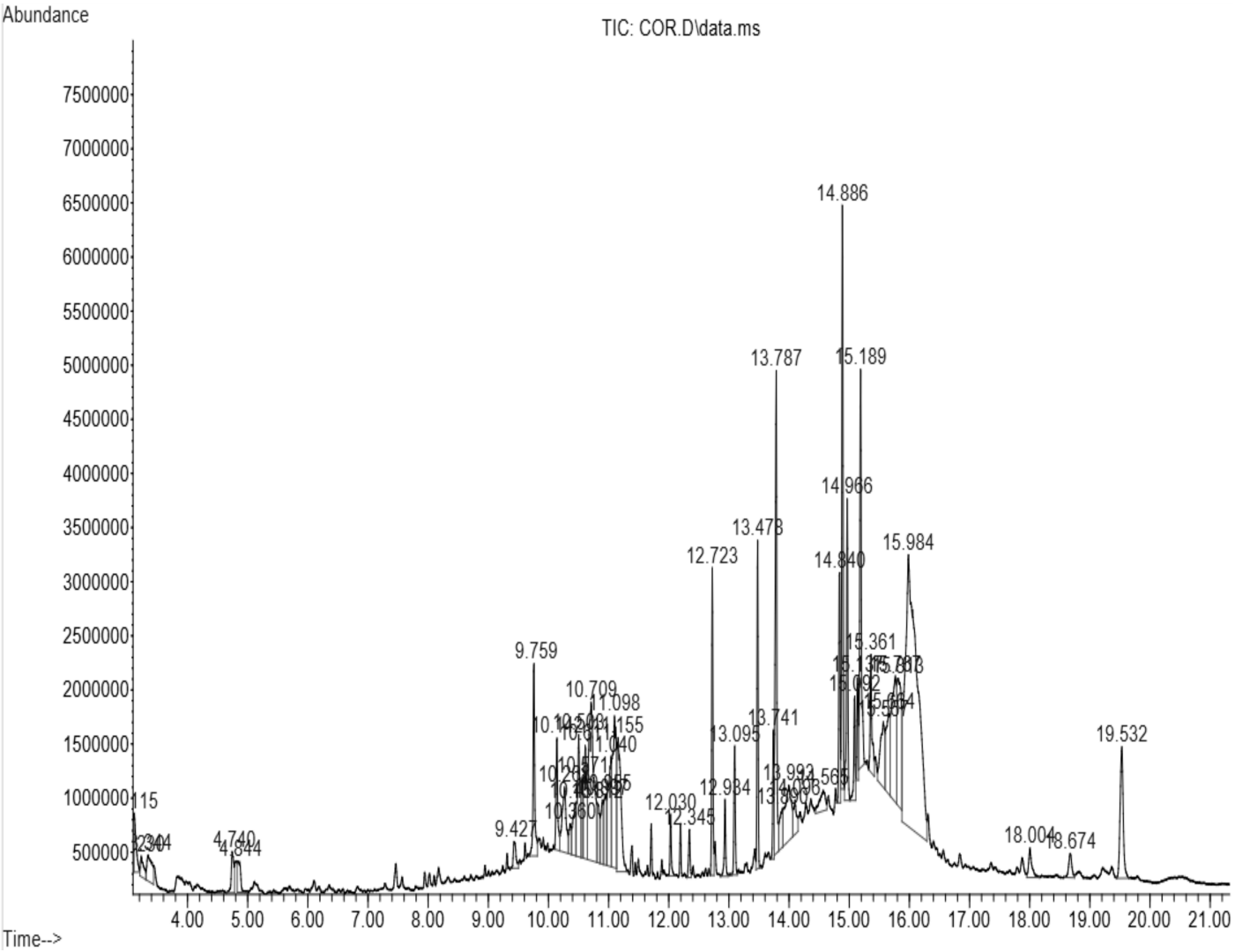

Fig. 3: GC-MS chromatogram of hydroethanol extract of *Corchorus olitorius* leaf showing the retention time (min) of the compounds in X axis and percentage (%) of peak area in the Y axis

Table 5: GC-MS chemical profile of various fractions of *Corchorus olitorius* leaf extract showing compound up to 0.7 % of total and above only

Peak No.	Compound	Molecular Formular	Molecular Weight (g/mol)	Retention Time (min)	% of Total
1	Phenol	C6H6O	94.03	3.344	0.83
2	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186.0	9.759	1.97
3	Dodecanoic acid	$C_{12}H_{24}O_2$	200.0	10.142	1.21
4	.beta.-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194.0	10.268	1.64
5	Pregna-5,17(20)-dien-3-ol, (3 beta .,17E)-	$C_{21}H_{32}O$	300.0	10.451	0.98
6	Diimidotricarbonic diamide	$C_3H_6N_4O_3$	146.0	10.503	2.11
7	Retinoic acid	$C_{20}H_{28}O_2$	300.0	10.571	0.83
8	1-Di(tert-butyl) silyloxypentane	$C_{21}H_{30}O_3Si$	358.0	10.611	1.92
9	Propanoic acid, 2-methyl-, octyl ester	$C_{12}H_{24}O_2$	200.0	10.709	5.34
10	Pregnan-20-one, 3-hydroxy-, 3.bet a.)-	$C_{21}H_{34}O_2$	318.0	10.812	0.89
11	Pregna-5,17(20)-dien-3-ol, (3.beta ,17E)-	$C_{21}H_{32}O$	300.0	10.897	1.03
12	Isosteviol acetate			10.955	1.10
13	Pregna-5,17(20)-dien-3-ol, (3.beta .,17E)-	$C_{21}H_{32}O$	300.0	11.040	2.15
14	3.beta.-Hydroxy-5-androsten-17-car boxylic acid	$C_{19}H_{30}O_2$	290.0	11.098	3.46
15	Isosteviol	$C_{20}H_{30}O_{33}$	318.0	11.155	3.28
16	Neophytadiene	$C_{20}H_{38}$	278.0	12.723	2.27
17	Neophytadiene	$C_{20}H_{38}$	278.0	13.095	1.08
18	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.0	13.478	2.36
19	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.0	13.741	1.08
20	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.0	13.787	4.65
21	1,2-Dicaprin	$C_{23}H_{44}O_5$	400.0	13.890	0.76
22	3-Dibenzofuranamine	$C_{12}H_9NO$	183.0	13.993	2.21
23	1-[p-Bromophenyl]-4-nitro-1,3-buta diene	$C_{10}H_9BrO_2$	241.0	14.565	0.73
24	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{18}H_{32}O_2$	280.0	14.840	1.86
25	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.0	14.886	4.50
26	Phytol	$C_{20}H_{40}O$	296.0	14.966	2.53
27	Methyl stearate	$C_{19}H_{38}O_2$	298.0	15.092	0.94
28	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.0	15.137	0.93
29	9,12,15-Octadecatrien-1-ol, (Z, Z,Z)-	$C_{18}H_{32}O$	264.0	15.189	3.89
30	Octadecanoic acid	$C_{18}H_{36}O_2$	284.0	15.361	1.29
31	Benzamide, N-cyclopropyl-4,5-diflu oro-2-methyl-	$C_{10}H_{11}NO$	161.0	15.567	1.75
32	1,2-Cyclohexanedicarboxylic acid, (2-chlorocyclohexyl)methyl isobutyl ester	$C_8H_{12}O_4$	172.0	15.664	1.97
33	1,3-Dicaprin	$C_{23}H_{44}O_5$	400.0	15.767	3.76
34	1,3-Dicaprin	$C_{23}H_{44}O_5$	400.0	15.813	3.29
35	3-Dibenzofuranamine	$C_{12}H_9NO$	183.0	15.984	20.32
36	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.0	19.532	2.52

Acetylcholinesterase inhibitory assay

The results as shown in Figures 4, 5, 7 and 8 are AChE inhibitory activities of both the extracts and their fractions and they are concentration dependent. Their IC_{50} reveal that the higher the inhibition percent the lower the IC_{50}. Their IC_{50} also reveal that fractionation of COR-CE produced fractions that are less active compared to the crude extract except the EA-COR (ethyl acetate fraction of *C. olitoriu*). And fractionation of XY-CE produced fractions that are more potent than the crude extract, XY-CE (Tab. 6).

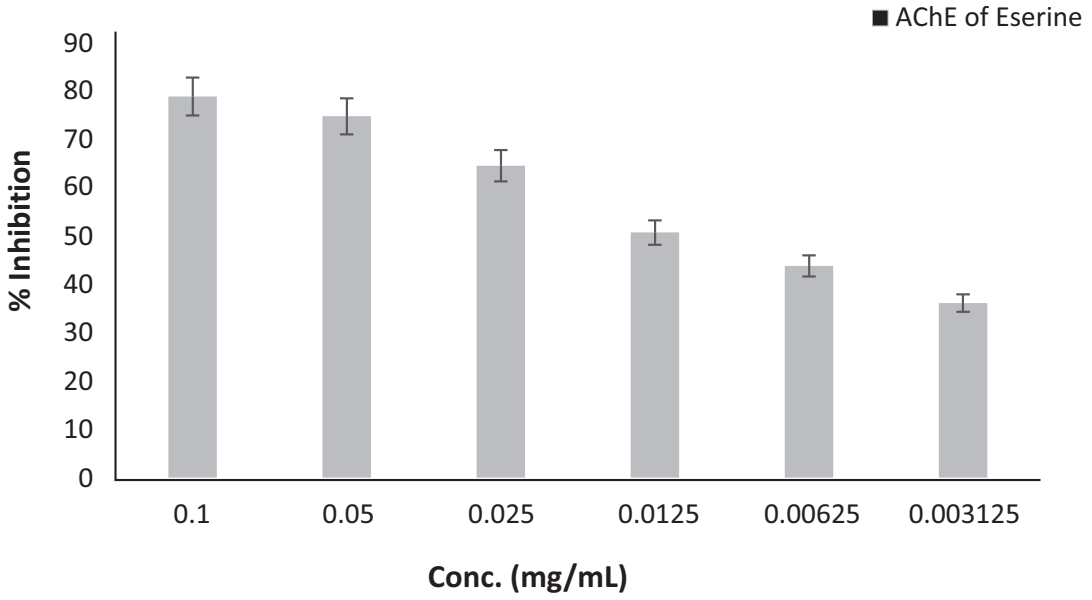

Fig. 4: Average AChE inhibition of the standard drug, Eserine

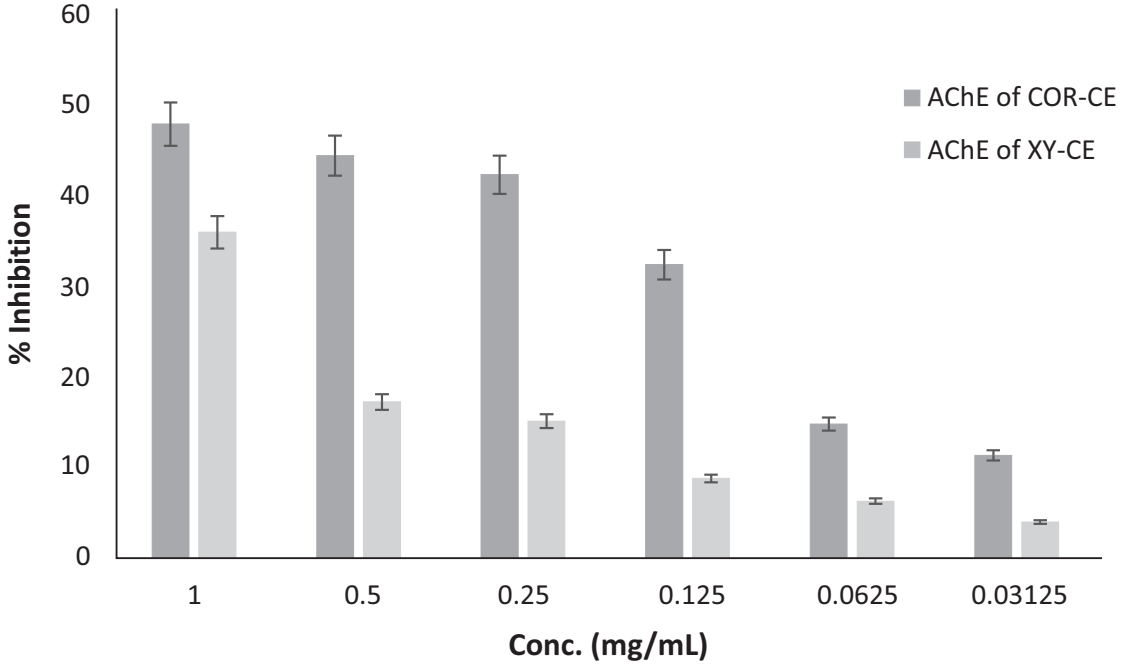

Fig. 5: Average AChE inhibition of the crude extracts of *X. aethiopica* fruit (XY-CE) and (COR-CE). Values are expressed as mean ± SEM (n =3); AChE: acetylcholinesterase

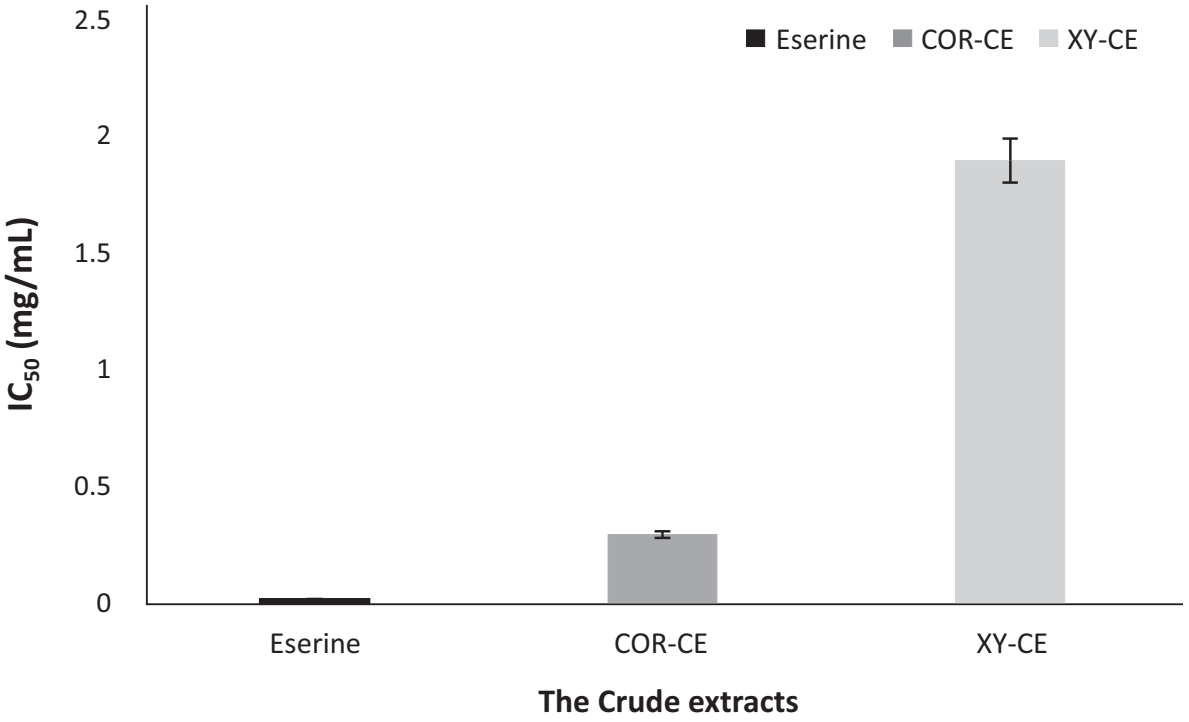

Fig. 6: The IC_{50} of *X. aethiopica* fruit (XY-CE) and *C. olitorius* leaf (COR-CE) hydro-ethanol crude extracts. IC_{50} represents the half-maximal inhibitory concentration.

The standard drug, eserine showed a better or much potent acetylcholinesterase inhibition, followed by COR-CE and XY-CE. * $p < 0.05$ compared to the eserine (positive control)

In summary: Eserine > cOR-CE > XY-CE

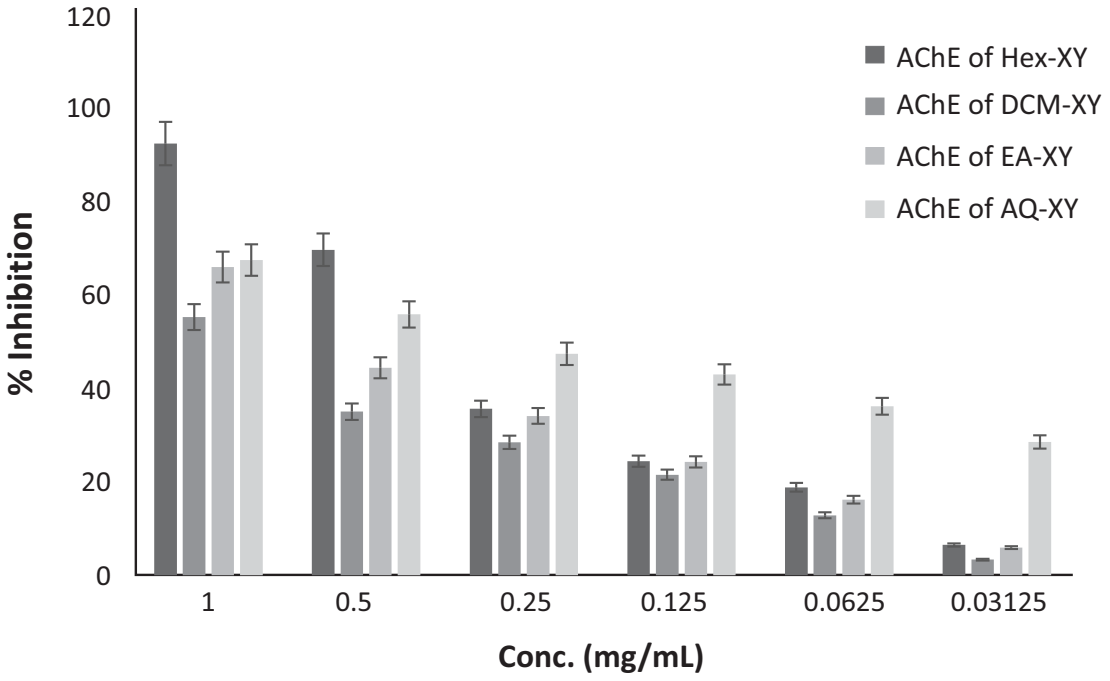

Fig 7: Average AChE inhibition of the solvent fractions of *X. aethiopica* fruit hydro-ethanol extract. Values are expressed as mean ± SEM (n =3); AChE: acetylcholinesterase

Hex-XY: Hexane fraction of *X. aethiopica* fruit crude extract
DCM-XY: Dichloromethane fraction of *X. aethiopica* fruit crude extract
EA-XY: Ethyl acetate fraction of *X. aethiopica* fruit crude extract
AQ – XY: Aqueous fraction of *X. aethiopica* fruit crude extract

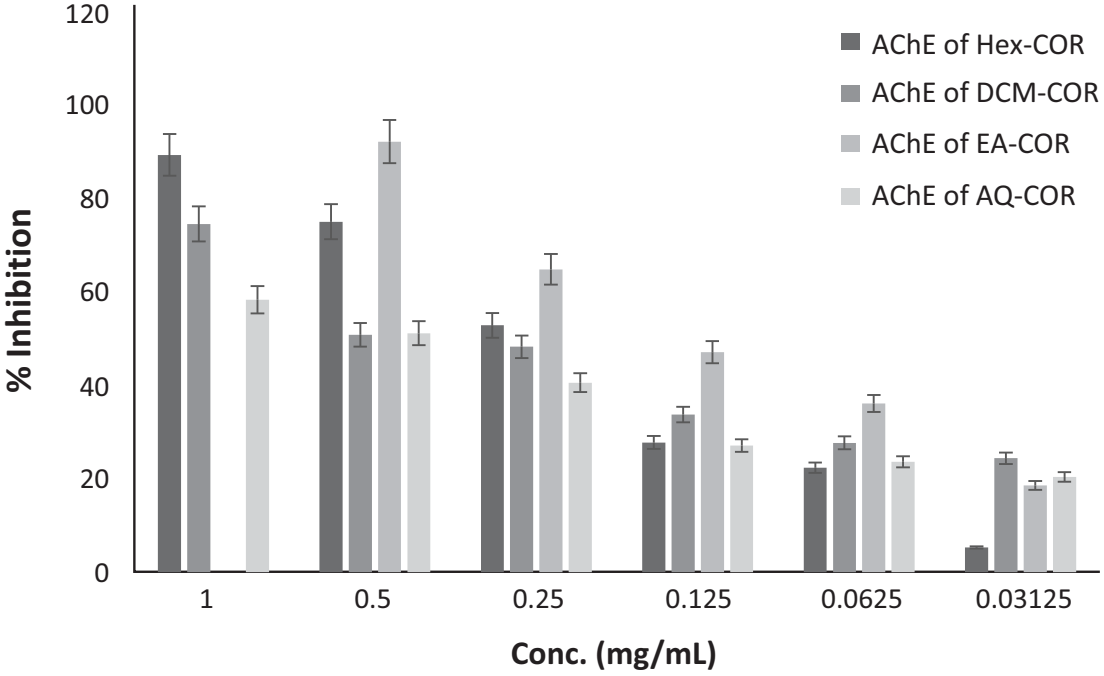

Fig. 8: Average AChE inhibition of the solvent fractions of *C. olitorius* leaf hydro-ethanol extract. Values are expressed as mean ± SEM (n =3); AChE: acetylcholinesterase

Hex-COR: Hexane fraction of *C. olitorius* leaf crude extract
DCM-COR: Dichloromethane fraction of *C. olitorius* leaf crude extract
EA-COR: Ethyl acetate fraction of *C. olitorius* leaf crude extract
AQ – COR: Aqueous fraction of *C. olitorius* leaf crude extract

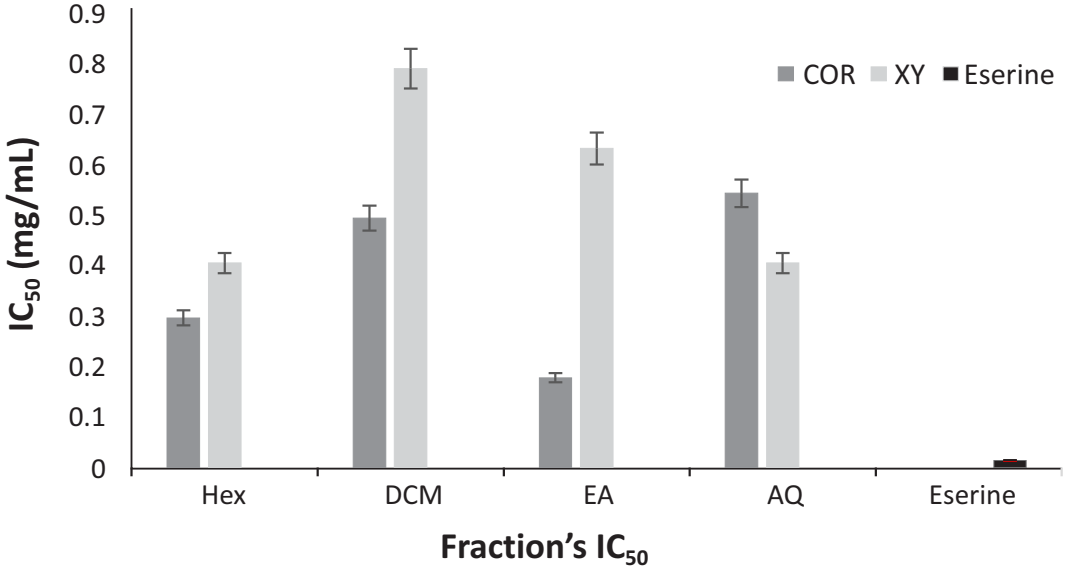

Fig. 9: The IC_{50} of *C. olitorius* leaf and *X. aethiopica* fruit crude extract solvent fractions. IC_{50} represents the half-maximal inhibitory concentration

For COR-CE fractions activity profile:

EA-COR >Hex-COR > DCM-COR > AQ-COR

For XY-CE fractions activity profile:

Hex-XY = AQ-XY > EA-XY > DCM-XY

Table 6: Anticholinesterase inhibition IC_{50} mg/mL of solvent fractions and the standard drug (positive control).

Crude/Fractions	COR IC_{50} mg/mL	XY IC_{50} mg/mL	Eserine IC_{50} mg/mL
CE	0.29 ± 0.00773[a]	1.89 ± 0.040[e]	-
Hex	0.30 ± 0.01[a]	0.41 ± 0.02[c]	-
DCM	0.55 ± 0.08[b]	0.80 ± 0.01[d]	-
EA	0.18 ± 0.01[a]	0.64 ± 0.08[d]	-
AQ	0.55 ± 0.08[b]	0.41 ± 0.12[c]	-
Eserine			0.014 ± 0.00

Results are expressed as mean ± SEM of triplicate experiments. Average values down each row with different superscript letters are significantly different ($P < 0.05$). IC_{50} represents the half-maximal inhibitory concentration. CE represents the crude extract.

CE: Crude extract; Hex: Hexane fraction;
DCM: Dichloromethane fraction;
EA: Ethyl acetate fraction;
AQ: Aqueous fraction

The various fractions of the XY-CE hydro-ethanol extract showed the least potency as indicated by their high IC_{50} compared to the same fractions of COR-CE (Fig 9 and Tab 6).

The lowest IC_{50} value of COR-EA (ethyl acetate of COR-CE, 0.18 mg/L) showed that it had the highest potency compared to the other fractions across the fractions from both the XY-CE and the COR-CE extracts. The potency of XY-CE increases on fractionation (Fig. 7) while the fractions of COR-CE showed lower potency as seen in higher IC_{50} than that of CPR-CE except that of EA-COR.

DISCUSSION

Plants have served as a source of new pharmaceutical products and inexpensive starting materials for the synthesis or semi-synthesis of some known clinically active drugs. Plant materials consist of rich and diverse classes of bioactive compounds with markedly different physiological and chemical structures. The efficacy of herbal preparation is dependent on extracting the right phytoconstituents. The compounds determine the biological activities of the plants and when isolated and studied can act as "leads" in synthesis of other functional drugs. The traditional preferential use of only alcohol and alcohol mixed with water in preparation of herbal products may be explained in the ability of the solvent to extract both polar, mid polar and non-polar compounds.[29,30] The phytochemical study of the 2 food-herb plants, XY and COR extracts revealed the presence of various secondary metabolites such as the alkaloids, terpene, saponins, flavonoids and so forth (Tables 2 and 3) while Tables 4 and 5 showed the identified compounds (carboxylic acids, esters, phenolic compounds and fatty acids) by GC-MS process. Phytochemical analysis showed that XY-CE and COR-CE contain alkaloids, phenolic compounds and other secondary metabolites however, the alkaloidal content of the XY-CE seem to be lower compared to that of the COR-CE based on the quantity of precipitate obtained using alkaloid reagents.[31]

Alkaloids have been identified as key compounds that exhibit the ability to inhibit the action of AChE. However, many experimental studies have documented the neuroprotective potency of other secondary metabolites such as the polyphenols, flavonoids and saponin contained in plant extracts.[32] And their ability to delay and prevent brain neuronal modulator depletion or injury

have also been demonstrated and reported.[33] The higher content of alkaloid seen in the COR-CE may reflect the differences seen in the cholinesterase inhibitory profiles of the two (2) crude extracts. The two extracts possess inhibition activity against AChE enzyme, however XY-CE has less or significantly ($p < 0.05$) less potency than that of the COR-CE as seen in Fig. 5 and Fig. 6.

Authors, Okereke *et al.*[34] and Okagu, *et al.*[35] have at different times reported the identification of 15, 58 and 26 phytoconstituents respectively from methanol extract of XY. In 2018, Tegang *et al.*[36] was able to identify 90 compounds from the essential oil of XY fruit from Cameroon. The XY-CE spectrum of the study showed 53 peaks, representing 53 identifiable phytocompounds (Tab. 4). The observed discrepancies could be from the source of the raw material and quality of solvent used for extraction, extraction method and the GC-MS equipment and library used. The environmental factor such as the soil and climatic difference could be a contribution factor towards the differential differences as reported by Okagu *et al*.[35] Thus, the essence of quality control assays after receipt of raw materials for compounding of herbal products. Phytocompounds identified in our study are known compounds and some of their pharmacological activities are available in the literature reports. Some compounds identified from XY hydromethanol extract include the ?-terpineol (methane monoterpenoids), naphthalene (polycylic aromatic molecule), kaur-16-ene (diterpenoids), octanoyl chloride. These named compounds have been reported to possess neuroprotective potential and possess anticonvulsant, sedative, anti-inflammatory and hypnotic activities. The octanoyl chloride is used in synthesis of a therapeutic agent for the management of Alzeimer's disease.[37]

Neophytadiene, a diterpene, was one of the phytocompounds identified from COR-CE. It has also been found in many plants such as the Crataeva nurvala and Blumea lacera.[38] Neophytadiene has been documented to exhibit/ exerts anxiolytic-like and anticonvulsant activities with the probable participation of the GABAergic system.[38] Isosteviol is another compound contained by COR-CE and known to possess neuroprotective both in vivo and in vitro documented experiments.[39] Retinoic acid is a derivative of vitamin A and has been documented to play an important role in the maturation, development and survival of neural cells. It has been proposed as a therapeutic option in the management of some neurodegenerative diseases, such as Parkinson's disease (PD).[40]

The presence of free radicals in the body has been linked to many chronic disease states. They can be precluded, mopped, or reduced by antioxidant and there by aid the control of such diseases. A lot of researchers have reported on the antioxidant profile of neuroprotective medicinal plants or moiety they studied. There seem to be a relationship between neuroprotective properties and antioxidant content of some plants.[41,42] This could be the ability of antioxidants to repair/protect against the accumulated effects of oxidative and inflammatory stress and possible ability to promote nerve cell regeneration or restore cellular oxidative damages. In our study, the eserine showed a significant ($p < 0.05$) greater antioxidant effect and a much higher AChE inhibitory effect than the XY-CE and COR-CE extracts. While COR (extract and fractions) showed a better and significant ($p < 0.05$) free radical scavenging (antioxidant) and AChE inhibitory effects than XY extract and fractions. However, our observation was not in line with Nwidu et al.[43] documented experimental reports that indicated no correlation/relationship between antioxidant ability and AChE inhibitory capability.

Acetylcholine (ACh) and butyrylcholine (BCh) are important neurotransmitters responsible for the transmission of impulses and function of the memory.[44-46] These neurotransmitters can be broken down by enzyme AChE resulting to cholinergic deficits, cholinergic neurotransmission termination and neurological dysfunction and degenerative diseases.[47] Neurocognitive dysfunction is a common manifestation in many neurological and chronic neurodegenerative diseases and presenting as learning and memory impairment, in many chronic diseases of the nervous system.[48] The trending theory and practice in the management of neurodegenerative diseases like Alzheimer is introduction of cholinesterase inhibitors (AChE inhibitors)[49] or replacement or elevation of acetylcholine in the brain. Many plants have been investigated for their ability to inhibit the activity of AChE inhibitors and some have shown to be good cognitive and memory enhancers.

The capacity of the crude extracts of XY fruit and COR leaf crude extracts and their fractions to inhibit AChE is shown in Figures 5 and 6. All the extracts and the fractions are showed to possess the ability to improve cholinergic neuronal function by inhibiting the activity of brain acetylcholinesterase. This observation is in line with previously reported potential neuroprotective effects of many medicinal and food plants that have shown their ability to inhibit cholinesterases' activities.[50-53] The report

of Sulaimon *et al.* (2020)[59] showed a lower value of IC_{50} for XY essential oil, 1.21 mg/mL, indicating that extracting the oil would be more potent that using the whole fruit.

The finding of this study shows that the AChE inhibitory activity of COR-CE is significantly ($p < 0.05$) stronger than that of XY- CE (Fig. 5). Meanwhile that of eserine is also significantly ($p < 0.05$) better than that of COR-CE as seen by their IC_{50} (Fig. 6). The lower the value of IC_{50}, the better the AChE inhibitory effect. This differences in efficacy may be as a result of effectiveness of eserine or due to the nature of eserine being a pure compound while the COR-CE was tested as a crude extract and as fractions. The same goes for the XY-CE that was tested as crude extract and fractions. Isolating the bioactive compound(s) from the test crude extracts may result in a more active bioactive compound that can serve as a lead compound, novel in effect and with less side effects.

The ethanolic extract of XY and its kaurene derivatives (example kaurenoic acid), xylopic acid, have been reported by Koomson *et al.*, 2022 to exert neuroprotection by improving exploratory learning, and various memory indices, spatial working and recognition in the behavioural tests.[55] This evidence based scientific experiment was done with the unripe fruits of XY. An ent-kaurene diterpene derived from XY extract has been reported to possess anti-inflammatory and antipyretic effects in animal models.[56,57]

Furthermore, the AChE inhibitory activity of the hexane, dichloromethane, ethyl acetate and aqueous fractions of the XY and COR were evaluated. The process of fractionating the crude extract of XY into various polarity fractions resulted in fractions with improved AChE inhibition. Hexane and aqueous fractions of XY (Fig 9) with IC_{50} of 0.41 mg/mL gave the best AChE inhibitory activity for the XY extracts. This showed that the bioactive compounds that exhibit the ability to protect the neurons are likely accumulated within the high polar and high non-polar compounds. The high or very polar compounds are mostly the phenolic compounds. It was found that ethyl acetate fraction of COR with IC_{50} value of 0.18 mg/mL was the most promising option for further investigation and possible isolation process. This may lead to the isolation of lead compounds for possible development to drugs that may be able to prevent the activity of the enzyme acetylcholinesterase in the brain and hence reduce the developmental progress of neurodegenerative diseases.[58] The obtained fractions of COR exhibited AChE inhibition significantly lower than the crude extract except EA-COR. This suggest that there is a synergy when the whole fractions combine as see in the whole COR-CE (Tab. 6). The possible best way to take COR as bioactive cognitive and memory enhancer is to take the whole leaf extract or take the EA-COR. The DCM-XY showed the least potency between the two (2) plant fractions as indicated by the high IC_{50} value of 0.80 mg/mL While XY-CE had the highest among all the tested samples. The suggested best way to take the XY for cognitive impairment effect would be to take it as fractions. Traditionally, it is being ground and used for cooking or soaked in water or alcohol with other herbs.

Thus, based on the AChE inhibitory effects as exhibited by these test natural product samples, they could be used to alleviate the symptoms of neurological disorders, improve memory, cognitive function and slow down the progress of the diseases. These are plant food and spices that are easily available, though seasonal in West Africa. The polyphenol content in vegetable food plants is known to be high and crosses the Blood-Brain barrier (BBB) to get to the brain.

This work was carried out with collaboration among all authors. Authors Orabueze, and G.E. Batiha designed the study. Authors I.C. Orabueze, F.O. Orabueze, E.A. Adeyinfa and T.M. Ghazali managed the literature search, carried out all experimental and data collection procedures. Authors I.C. Orabueze and G.O. Oludare supervised the work and wrote the protocol. All the authors were involved in results evaluation. The manuscript preparation was done by I.C. Orabueze and G.A. Asare. Final approval of manuscript was done and accepted by all the authors.

CONCLUSION

The results suggested that XY fruit and COR leaf and their partition solvent fractions possessed the ability to protect the neurons, improve cognitive index, memory and learning ability. And thus, these food herb plants could be utilized as possible dietary intervention in the management of age-related neurodegenerative diseases (especially AD and PD) and improve post malarial infection cognitive dysfunction in children. The two fractions, EA-COR (IC_{50} - 0.18 mg/mL) and Hex-COR (IC_{50} - 0.30 mg/mL) and Hex-XY (IC_{50} - 0.40 mg/mL) can be further evaluated in animal models and subjected to isolation of bioactive compounds,

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